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Biases in extracting Collembola through Tullgren funnel

BY

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I. — INTRODUCTION

According to EDWARDS and FLETCHER (1971), who sent out questionnaires to soil zoologists and ecologists all over the world, 74 % of workers use some form of Tullgren funnel and 82 % of them use a relatively standard type, usually with electric light as a heat source.

However, it has been suspected that the results with Tullgren funnels might not reflect the real composition of the fauna in natural conditions. The efficiency of this technique is hard to evaluate, for the dead bodies remaining in the sample can hardly be searched out. By direct comparison with hand-sorting, MACFADYEN (1955) reported that the rather primitive type of Tullgren funnels extracted about 35 % of the collembolan fauna. It is generally supposed that the standard type of Tullgren funnels extract only 10 to 30 % of Collembola and 30 to 40 % of Acarina from the soil samples (NIIJIMA *et al.*, 1968). Recently, WILLARD (1972) reported that only 20.8 % of collembolan assemblage as a whole was extracted with Salt-Hollick method (SALT and HOLLICK, 1944) which is one of the flotation methods and generally regarded to be better than Tullgren type funnels in extraction efficiency. However, few studies have been made to estimate a real, natural population by correcting the number of individuals with regard to such insufficient extractions.

It has been suspected also that Tullgren funnels might give biased extraction depending on differences in the size of individuals in a given population (HAARLØV, 1962). Once I have tentatively compared the funnel extraction with the hand-sorted sample separately for roughly divided size classes of Collembola. The extraction of smaller individuals was seen to be slightly less efficient, but no previous studies have reported such a tendency. In this study I took aim at three points: (1) Evaluation of the extraction efficiency of Tullgren funnel for individual species of Collembola, compared with hand-sorted

sample; (2) Detailed examination of the size-specific tendencies in Tullgren funnel extraction; and (3) Estimation of individual number in a natural population of Collembola.

II. — METHODS

The sampling was carried out in a subalpine coniferous forest at « Otanomosu-no-Taira » in Shiga Heights, central Japan, on November 16th, 1971, when the snow covered the forest floor about 30 cm thick. First, snowcover was completely removed by hand at the sampling site, and a soil block about 30 × 30 cm with litter layer was taken 5 cm deep, while 10 quantitative soil samples were also taken by using tin samplers (5 × 4 × 5 cm).

In the laboratory, the 10 smaller samples were individually weighed. The mean weight was 76 g, so the weight of one sample was regarded as 80 g to make the calculation easy. The larger sample was thoroughly but gently mixed up to homogenize the soil and litter without exerting high pressure upon it. Then the homogenized sample was quickly divided into four subsamples of roughly equivalent quantity. From each subsample, the 80 g soil for Tullgren funnel and the 10 g soil for hand-sorting were alternatively taken five times after softly stirring the subsample by hand each time in order to maintain the homogeneity as far as possible. Consequently the « samples » amounted to forty; twenty each for Tullgren funnel and hand-sorting, respectively. Of the twenty samples for hand-sorting, however, only ten samples were randomly selected for practical treatment.

The « samples » for Tullgren funnel were illuminated for 42 hours with the standard type apparatus described in KIKUZAWA *et al.* (1967). The animals extracted into 70 % alcohol were mounted in total preparations with Hoyer's mounting medium (Tamura, 1974). The « samples » for hand-sorting were also preserved in 70 % alcohol. Later, the « samples » were directly observed under a stereoscopic microscope of high power and all the animals found except Protozoa were mounted all together in microscopic preparations also with Hoyer's solution. The preparations after hardening were microscopically examined for identification and counting the number of animals at specific level for Collembola and at higher level for the others. Further, *Folsomia octoculata*, a collembolan species, was individually measured for the body length using the profile projector-curvimeter method (TAMURA, 1974).

III. — RESULTS

1. Extraction efficiency of Tullgren funnel.

The numbers of individuals extracted or sorted were converted into density per m^2 in Table I. It is clear that the extraction with Tullgren funnel gave results differing from the natural * population makeup represented by hand-sorting, and the amount of difference was not the same for different animal groups. The main cause of these complex differences is probably due to the insufficient extraction through Tullgren funnel. Among the « Tullgren fauna », Collembola and Acarina were overwhelmingly dominant. But among the hand-sorted « natural fauna », Harpacticoida and Enchytraeidae were the most abundant, followed by Collembola and Acarina. The principle animals whose number were of the same order of magnitude with both methods of extraction were *Folsomia octoculata* (Collembola) and Oribatei (Acarina).

Individuals of each animal group per sample (80 g soil) in the two extractions were calculated in Table II for the groups with the densities over 1,000

* Validity of hand-sorting may not be always absolute, but extraction by hand-sorting gives numbers which should approximate the natural figure at least for Collembola, taking account of their enlarged size under a stereoscopic microscope.

TABLE I

Number of individuals converted into density per sq. m in both extractions

Animal group	T-funnel	H-sorting
Collembola	16,550	103,600
Onychiurus flavescens	1,225	29,600
Onychiurus longisensillatus	325	2,800
Tullbergia krausbaueri	25	0
Hypogastrura denisana	1,475	5,200
Friesea japonica	1,125	6,000
Neanura sanctisebastiani	300	0
Isotoma olivacea	25	800
Isotoma carpenteri	0	1,600
Folsomia octoculata	11,325	50,400
Pteronychella perpulchra	425	6,000
Tomocerus varius	0	400
Pseudoscorpiones	0	400
Prostigmata	475	29,200
Mesostigmata	1,600	6,400
Oribatei	13,125	49,200
Harpacticoida	0	252,000
Nematoda	0	462,400
Enchytraeidae	25	179,200
Rotifera	0	6,400
Diptera (larva)	575	6,400
Coleoptera (larva)	275	8,400
Tardigrada	0	20,800
Psocoptera	0	800
TOTAL	32,625	1,125,200

TABLE II

Mean number of individuals with standard deviation per 80 g soil sample in both extractions and their ratio (T/H) for each animal group exceeding 1,000 individuals per sq. m in Tullgren extraction

Animal group	T-funnel	H-sorting	T/H (%)
Collembola	33.10 ±10.53	207.20 ±88.40	15.97
Onychiurus flavescens	2.45 ± 2.11	59.23 ±33.60	4.14
Hypogastrura denisana	2.95 ± 1.47	10.41 ±10.33	28.37
Friesea japonica	2.25 ± 1.80	12.08 ±11.45	18.75
Folsomia octoculata	22.65 ± 8.55	100.79 ± 8.35	22.47
Mesostigmata	3.20 ± 1.03	12.81 ±10.85	25.00
Oribatei	26.25 ±12.30	98.42 ±49.71	26.70

individuals per m² in Tullgren extraction. The collembolan assemblage as a whole was extracted with an efficiency (T. funnel/H.-sorting) of 16.0 %. However, the efficiency was remarkably different among collembolan species. The species regarded to be relatively tolerant of desiccation such as *Hypogastrura denisana* and *Folsomia octoculata* ranged between 20 to 30 %, within which the efficiencies of Mesostigmata and Oribatei also fell. But the

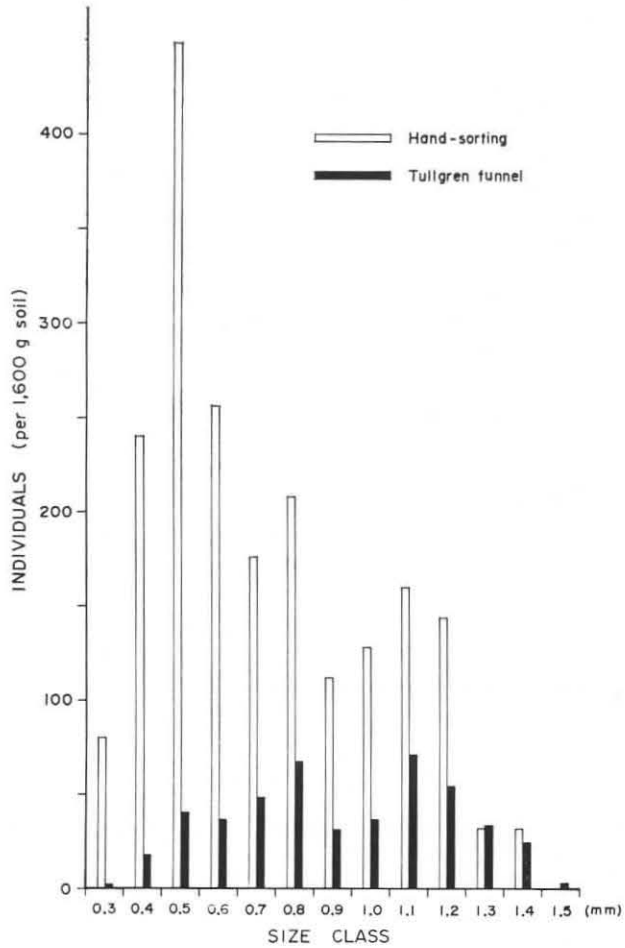

FIG. 1. — Frequency distribution of each size class at intervals of 0.1 mm of *Folsomia octoculata* in both extractions per 1,600 g soil sample.

species susceptible to desiccation such as *Onychiurus flavescens* (4.1 %) were remarkably difficult to extract. *Friesea japonica* (18.8 %) lay between the two types in the efficiency. According to FORSSLUND (1948), collembolan assemblage showed 24 to 27 % extraction efficiency in the Tullgren funnel. This suggests that the funnel used in the present study did not function abnormally.

2. **Relationship between body length and extraction.**

Folsomia octoculata, the overwhelmingly dominant species in the area surveyed, was selected as material to examine any biased trend with respect to body size differences. All specimens obtained by the two extractions were measured for the body length. The results are shown in Fig. 1 as a histogram. From glance at the figure, it is seen that the Tullgren funnel is remarkably inefficient as a whole and particularly in extracting smaller individuals. In other words, there is a distinct bias size-dependent in extraction through the Tullgren funnel. Accordingly the ratio of hand-sorting/Tullgren funnel

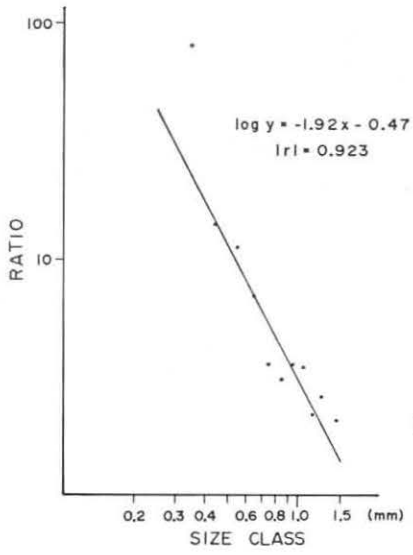

FIG. 2. — Distribution of H/T ratio against size class of *Folsomia octoculata* on double logarithmic scale.

(H-T ratio) for each size class was calculated on the basis of the number of individuals corrected for same weight of the soil sample (1,6000 g) in both extractions. When the relationship was plotted on double logarithmic paper, a negative linear relationship was recognized excluding the minimum size class (Fig. 2). No more than a single specimen was extracted in the minimum size class by Tullgren funnel in this experiment, so that it was ignored in the least square method to describe the relationship. It is clear that the ratios became larger as the body size of Collembola diminished.

The equation obtained is,

$$\log y = -1.92 \log x + 0.47, \text{ or}$$

$$y = \frac{2.95}{x^{1.92}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

, where y is the H-T ratio when the body length is x.

3. Estimation of a natural population.

In the experiment mentioned above executed in November, it was suspected that the animals, especially smaller ones, were harder to extract than in other seasons because they became inactive with onset of winter. TAMURA *et al.* (unpublished) conducted an experiment * with the same procedure at the same area in August (1972), which seems to be season of greatest activity. The result showed also a negative exponential relationship between size and H-T ratio, expressed by

$$y = \frac{2.10}{x^{1.76}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

The equations (1) and (2) are fairly similar to each other. But the latter (2) has a gentler slope and a smaller asymptote to the abscissa, suggesting a higher efficiency of Tullgren funnel in extracting animals, especially smaller ones, in the warmer season.

From the comparison of the equations (1) and (2), the ratio (y_i) of the number of individuals of a hand-sorted « natural population » to those through Tullgren funnel for a given body length (x_i) can be generally denoted follows:

$$y_i = \frac{a}{x_i^{\,b}}$$

, where a and b are constant. Presumably this relationship may be also applied to the species other than *Folsomia octoculata,* provided reasonably ample fluctuations in a and b are permitted. Then, a and b will probably vary with the species examined, seasons in which the survey was carried out, types of funnel used, types of soil sampled, etc. The number of individuals of size x_i in the « natural population » (Y_i), when those passed through the funnel are n_i, may be calculated as follows:

$$Y_i = \frac{a}{x_i^{\,b}} \cdot n_i \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Consequently the number of total individuals of a natural population (N) is given by

$$N = \Sigma \frac{a}{x_i^{\,b}} \cdot n_i.$$

With special reference to *Folsomia octoculata* in the present study area, the specific « natural population » at a given time is estimated to lie within the following range:

* Carried out by Drs. J. AOKI, K. NIIJIMA, Mr. K. FUKUYAMA and H. T. Hand-sorting was performed by all of the four members. Preparation, identification, measurement of body length and calculation for Collembola were all done by H. T.

$$\Sigma \frac{2.10}{x_i\ 1.76} \cdot n_i \leqslant N \leqslant \Sigma \frac{2.95}{x_i\ 1.92} \cdot n_i.$$

If the forest floor is covered with snow, then the N may approach the right side, while if there is no snow cover, the N will be near the left side. However, the positions of N the intermediate seasons can not be determined at the present.

4. Application of the correction equation to some model « Tullgren populations ».

Hyperbolic relationship between the body size of Collembola and H-T ratio means that if a population through Tullgren funnels at a given time has a population structure skewed to the smaller sizes in the number of individuals, then the corresponding « natural population » must be larger than another population with the same number of individuals skewed to the larger sizes. Therefore, it is an important prerequisite for correct estimation of the natural population to determine the pattern of population structure of a given « Tullgren population ».

If there is a hypothetical population through Tullgren funnels with the density of 10,000 individuals per sq. m divided into ten size classes, then several models representing typical population structure can be constructed (Fig. 3). These models, all composed of the same number of individuals, are each characterized as follows:

I. Pattern with normally distributed size classes with a mode at the middle;
II. Pattern skewed to the small-sized classes;
III. Pattern skewed to the large-sized classes;
IV. Pattern with bimodally distributed size classes with a break at middle;
V. Pattern with bimodally distributed size classes with a break toward larger sizes;
VI. Pattern with bimodally distributed size classes with a break toward smaller sizes;
VII. Pattern with main loss of individuals at later growth period;
VIII. Pattern with main loss of individuals at early period of growth; and
IX. Pattern with gradual loss of individuals at a constant rate throughout the period of growth.

Each model population was converted into the « natural population » for the number of individuals by using the correction equation (3).

In Fig. 4 (I-IX), the results were given as curve lines together with the corresponding model just mentioned. In all figures the top lines were always those corrected by Eq. (1) from the November data, the intermediate ones by Eq. (2) from the August data, and the bottom ones those of the respective model populations expressed with line drawing. Consequently the extent of natural population probably lies within the dotted area of each figure. In other words, the individuals within the open area correspond to those which failed to be extracted with Tullgren funnels. Throughout the figures remar-

kable discrepancies are seen between Tullgren model and corrected populations in the number of individuals as well as the pattern of population structure. The models with many individuals in small-sized classes (II, IV, V, VII, VIII and IX) differed considerably in number of individuals from the « natural population ». On the contrary, the models with more larger individuals (I, III and VI) do not deviate so far from the « natural populations ». Further the recorded and estimated population patterns are very different in the

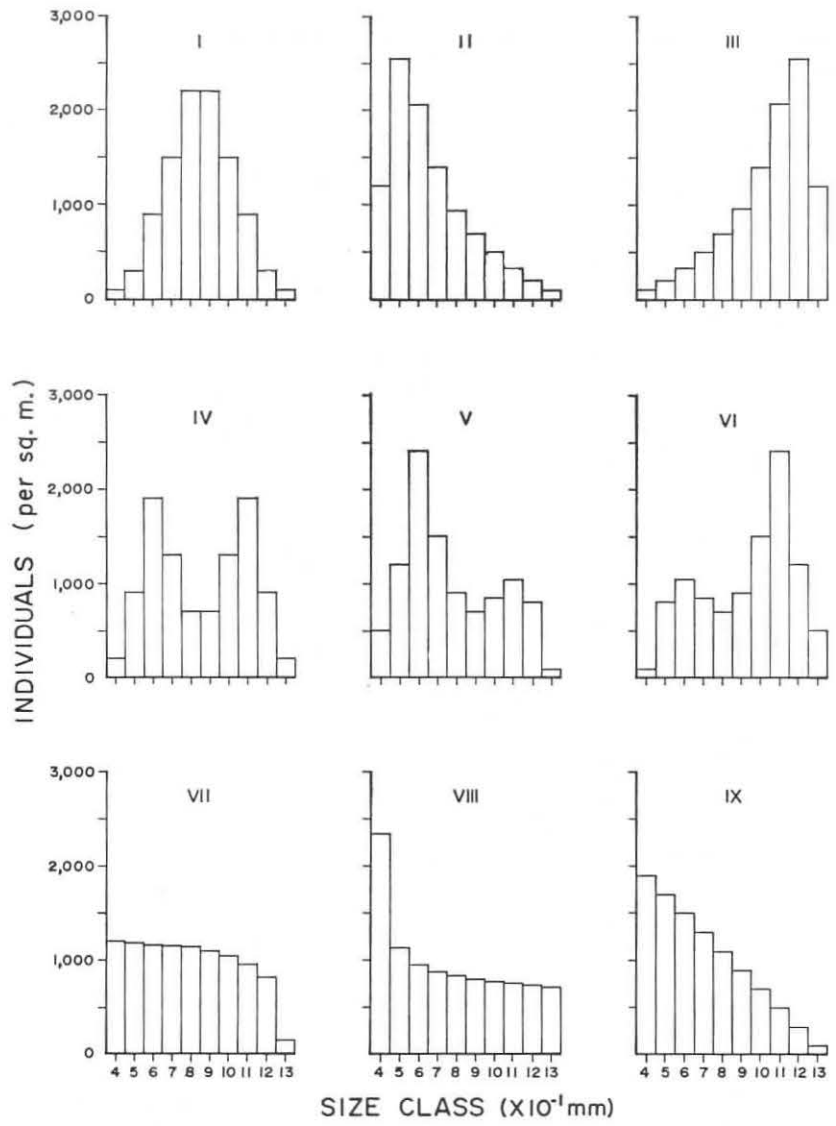

FIG. 3. — Some model Tullgren populations (I-IX), each with respective population structure of 10,000 individuals per sq. m divided into 10 size classes.

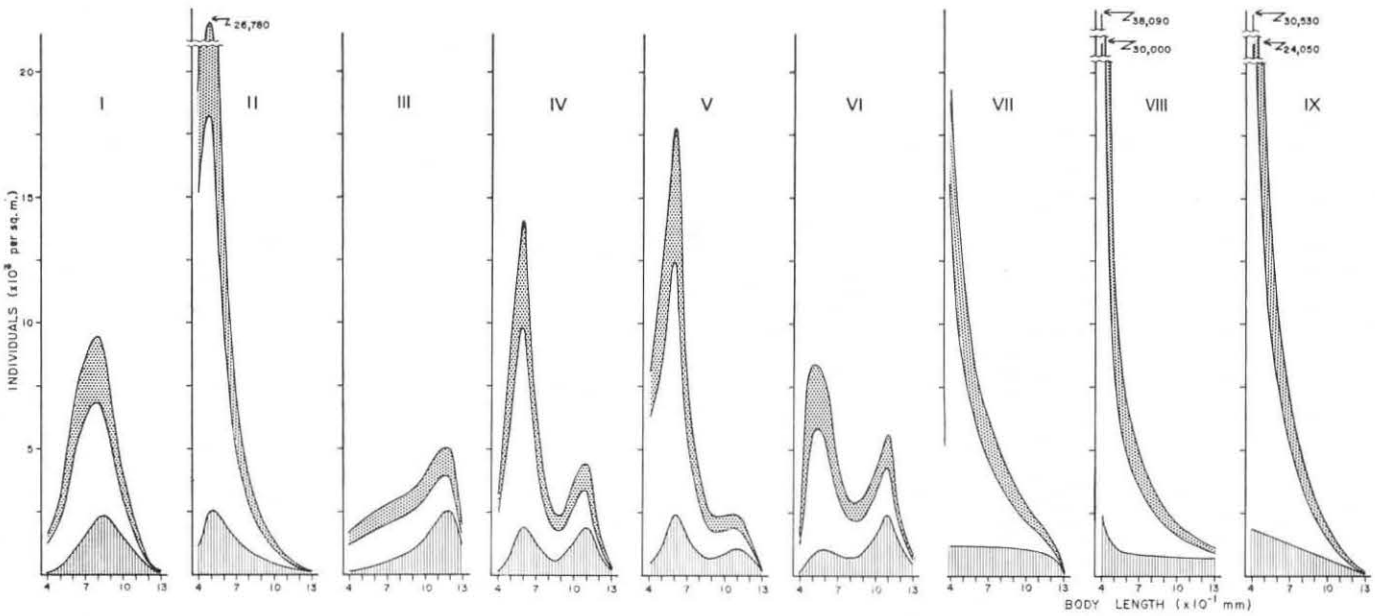

FIG. 4. — Line drawing representation of corrected model populations (I-IX) for number of individuals in each size class; top line corrected with November equation, intermediate with August equation, and bottom line drawing rearrangement of corresponding model (for details see text). Dotted area means the possible range of the natural population and striped area quantum of model Tullgren population.

former models just mentioned, while not so much in the latter models. It is noticeable, however, that the position of the mode of population composition was almost unaffected even in the models II, IV and V as well as in those dominated by larger size classes (I, III and VI).

5. **Application of the correction to a previous record.**

The correction is also applicable to the data of *Folsomia octoculata* at Hidaka-Mombetsu, Hokkaido, by TAMURA *et al.* (1969). The original data had been given as the relative abundance of the total number of individual without the body size measurement. The original data given with a slight modification (bottom in Fig. 5) suggest univoltinism with the breeding season during June to September followed by monotonous decrease. The population structure at each sampling time is assumed as follows (cf. III-4): model I in May; IV in July; V in September; I in November; and I in January. The natural popu-

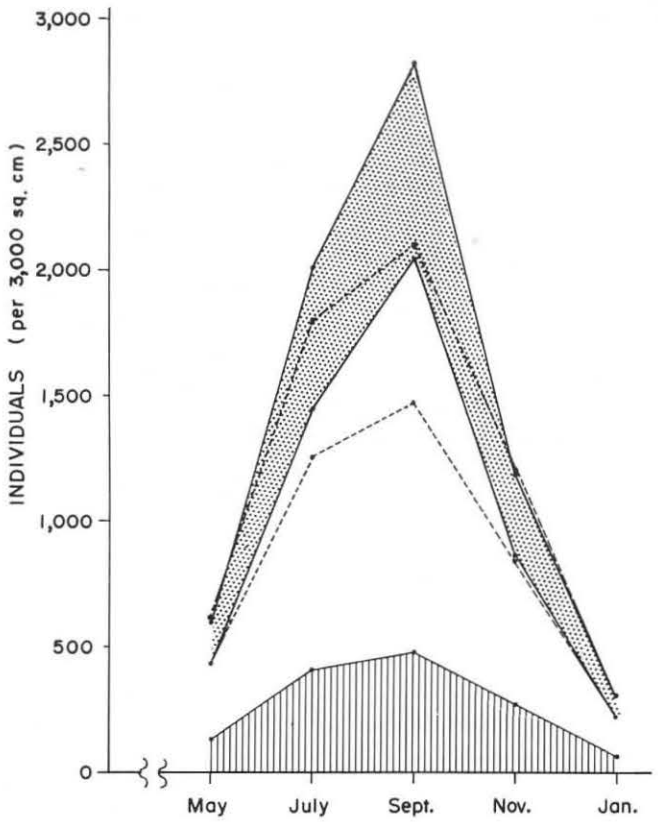

FIG. 5. — Phenological trend of the natural population of *Folsomia octoculata* at Hidaka-Mombetsu, Hokkaido, estimated from the original data by TAMURA *et al.* (1969); dotted area indicates the range of the natural population, striped area quantum in the original data. Area surrounded by broken lines is population range by simple correction (for details see text).

lation is estimated by correcting the number of individuals divided into each size class in proportion to the population structure of corresponding population (Fig. 5). Further the original data were simply multiplied by reciprocals of the extraction efficiencies for the population of *Folsomia octoculata* in November (22.47 %) and August (30.74 %) as a whole regardless of size, and the results were added to the figure. In the present example, the absolute number of individuals of the natural population was considerably different from the original figure, but the trend pattern was quite the same with each other. The range between two simple corrections multiplied by reciprocals of the extraction efficiencies partly corresponds with that of the natural population.

In conclusion, the « Tullgren population » deviates so much from the natural population, that it is very dangerous to regard the number of individuals obtained through Tullgren funnels as the natural population without corrections, though the « Tullgren population » can serve to some extent as basis for comprehension of the life cycle and the phenological pattern of a given population of Collembola, provided some adequate corrections are made.

IV. — **DISCUSSION**

For the soil animals, especially for the group of mesofaunal animals or smaller ones, direct sorting or hand-sorting, very simple in theory, is virtually impracticable from the viewpoint of both time and labour economy. Among a number of indirect methods devised for extracting animals the funnel-type extractor using electric heat as stimulus prevails in the world. But the funnel method had always a serious defect in the extraction efficiency. The problem has been a puzzle to soil zoologists. In the experiment given above, too, a remarkably low extraction efficiency was revealed for Collembola as well as some other groups as cited in the beginning of the text. In such circumstances, new or modified extraction methods are being proposed one after the other to get higher efficiency. But as a result of these modifications the apparatus has gradually become more complicated and more expensive. For example, the Macfadyen high gradient canistar extractor (MACFADYEN, 1962; BLOCK, 1966) and the Macfadyen air-conditioned funnel extractor (MACFADYEN, 1962) showed better results (nearly perfect according to MACFADYEN himself, but with a suspicious doubt according to HEALEY (1971)), but both are too expensive to be used by many researchers including beginners. Moreover in spite of all it seems impossible to attain 100 percent efficiency. Nevertheless, one of the means of settling the trouble will be obtained by establishing a method to convert the « Tullgren population » into the « natural population », though it is undoubtedly important *sine que non* to use a more improved apparatus, with due regard to labour, time and cost. If the extracting ability of Tullgren funnel is uniformly low regardless of the size of collembolan individuals, the natural population will be easily obtained by multiplying the total number of individuals of a given « Tullgren population » by reciprocal of extraction efficiency of the funnel used. However, in the present experiment, it was proved that the extraction efficiency varied with the size of Collembola. Recently TAKEDA (1973) examined the extraction efficiency of a dynamic method similar

to Macfadyen's high gradient canister extractor aginst a flotation method for each of eight roughly divided size classes of *Folsomia octoculata*. In his experiment the flotation method was employed as the contral. But the flotation method is not very successful (EDWARDS and FLETCHER, 1971) and may extract animals which died prior to the experiment, and exuviae (often mistaken for animal bodies dehydrated in alcohol from soil samples). Leaving aside this question, TAKEDA reported that only the first class was about six times less extractable in the high gradient funnel and the other seven classes were almost unbiased. Reading his text figure (Fig. 4), however, there appeared to be a slightly negative exponential relationship between size of the animals and the extraction ratio (flotation/canister).

Consequently it may be generally confirmed that the small-sized individuals are hard to extract compared with the larger, with some negative exponential relationship between the size of collembolan individuals and extraction ratio. Therefore, it seems risky to regard the « Tullgren population » as a faithful copy of the « natural population ». Further the « Tullgren population » simply multiplied by a reciprocal of the extraction efficiency of a given population as a whole is also insufficient to estimate the natural population in a strict sense, though it could give an approximation relatively nearer to the natural population than the « Tullgren population » itself. The most reliable approximation to real « natural population » will be given, all things taken together, by incorporation of the size parameter such as body length into estimation.

However, it is obviously impossible to examine the extraction efficiency of the Tullgren funnels used, for every instar or size class of collembolan populations in all surveys. Therefore, the extent of corrections must be determined depending upon the precision required by the purposes of surveys. From the present experiments, the following three categories may be proposed.

1) *Qualitative life history studies:* Patterns of the population structure of « Tullgren population » and « natural population » roughly agree with each other (cf. Fig. 3). The qualitative life history studies such as determination of the times of begining and end of recruitment, of the period of breeding and of the individual growth pattern, may require no corrections for the number of individuals.

2) *Studies on the phenological trend of a population:* Patterns of the seasonal fluctuation of « Tullgren population » and « natural population » are not so different from each other except the amplitude of population size (cf. Fig. 5). Correction for the extraction efficiency of the Tullgren funnel for a given population as a whole, regardless of size of individuals, may provide the appropriate estimate of the « natural population ».

3) *Studies on life table and productivity of a population:* These studies need the exact number of individuals for each instar or size class as the minimum requirement. Extraction efficiency of the Tullgren funnel for each instar or size class are required there, at least for the two periods of highest and lowest mobility throughout the year. The efficiencies in the intermediate seasons, if available, may produce a still more reliable estimate for population ecology.

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Summary

The extaction efficiency of a modified Tullgren funnel was examined for the samples taken form Otanomosu-no-Taira, Shiga Heights, in November, 1971, in a comparison with the hand-sorting. Regarding the results of hand-sorting as the « natural » fauna, the funnels used showed a very low extraction efficiency as follows: 16.0 % for the whole collembolan assemblage: 20 % to 30 % for *Hypogastrura denisana* and *Folsomia octoculata*; and 18.8 % and 4.1 % for *Friesea japonica* and *Onychiurus flavescens*, respectively. From examination on *Folsomia octoculata*, it was revealed that the deficiency of Tullgren funnels was more intense in small-sized individuals than in larger ones. The ratio of hand-sorting/Tullgren funnel (H-T ratio) for each size class at 0.1 mm intervals decreased exponentially with the increase of body size of individuals. Between the ratio *(y)* and the body length *(x)*, the following relationship was established.

$$y = \frac{2.95}{x\,1.92}.$$

The number of total individuals (N) of a natural population is given by

$$N = \Sigma \frac{a}{x_i\, b} \cdot n_i$$

where n_i is the number of individuals at x_i size class of the funnel extraction and a and b are constant.

Application of the correction equations to several model populations with different structure composed of 10,000 individuals per sq. m showed a remarkable divergence among models. The correction equations were also applied to a result on *Folsomia octoculata* already published. The « natural population » was largely different from the « Tullgren population » in population size. However, the patterns of phenological trend of both « populations » were fairly similar with each other. At any rate, it seems to be risky to regard the « Tullgren population » as the « natural population » itself or its justified approximation.

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